

CLAIMS

1. A method of converting terminal hydroxyl groups of oligonucleotides into phosphate monoesters, comprising of the steps of:

a) coupling of a phosphoramidite reagent to the terminal hydroxyl group of an oligonucleotide, wherein the phosphoramidite reagent is characterized by being a solid compound carrying two phosphate protective groups that are removable in one deprotection step together with the base protective groups of the oligonucleotide and carrying a reporter group attached to one of the protective group, and

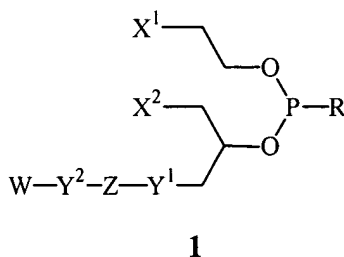
b) removing the phosphate protective groups.

2. The method of claim 1 wherein the phosphoramidite reagent carries two phosphate protective groups which are removable via β -elimination.

3. The method of claim 1, wherein the reporter group is released under conditions commonly applied in the detritylation step of the SPOS, and wherein its concentration is measured by photometrical means.

4. The method of claim 3 wherein the reporter group is a DMT group.

5. The method of claim 1 wherein the phosphoramidite reagent is represented by formula (1):



wherein

R is a dialkylamino group having the formula $-NR'R''$, wherein R' and R'' are independently selected from the group consisting of an alkyl group having from one to about ten carbons, or wherein R' and R'' together form a cyclic alkylene group having from two to up to twenty carbons which may or may not have additional alkyl

substituents attached to it and which may contain up to 3 heteroatoms selected from the group consisting of N, O and S included in the cyclic alkylene group;

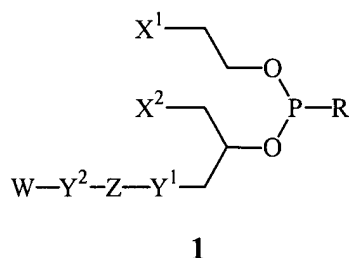
X^1 and X^2 are independently selected from the group consisting of nitrophenyl, cyano, alkylsulfonyl, aryl, and arylsulfonyl, wherein the alkyl moiety is selected from the group consisting of a branched or unbranched alkyl group having from 1 to 10 carbon atoms and the aryl moiety is selected from the group consisting of a phenyl group substituted with 0 to 5 substituents, wherein said substituents are independently selected from the group consisting of chlorine, fluorine, bromine, cyano and nitro.

Y^1 and the optional moiety Y^2 are independently selected from the group consisting of CH_2 , O, NH, $O(CO)$, $NH(CO)$, $O(CO)O$, $O(CS)O$, $NH(CO)O$, $NH(CS)O$, $NH(CO)NH$, $(CO)O$ and $(CO)NH$;

Z is an optional spacer unit selected from the group consisting of alkylene and oligoethylene glycolyl and combinations thereof, which may be unsubstituted or substituted; and

W is a nucleosidic moiety containing the reporter group.

6. A phosphoramidite reagent having the formula:



wherein

R is a dialkylamino group having the formula $-NR'R''$, wherein R' and R'' are independently selected from the group consisting of an alkyl group having from one to about ten carbons, or wherein R' and R'' together form a cyclic alkylene group having from two to up to twenty carbons which may or may not have additional alkyl substituents attached to it and which may contain up to 3 heteroatoms selected from the group consisting of N, O and S included in the cyclic alkylene group;

X^1 and X^2 are independently selected from the group consisting of nitrophenyl, cyano, alkylsulfonyl, aryl, and arylsulfonyl, wherein the alkyl moiety is selected from the

group consisting of a branched or unbranched alkyl group having from 1 to 10 carbon atoms and the aryl moiety is selected from the group consisting of a phenyl group substituted with 0 to 5 substituents, wherein said substituents are independently selected from the group consisting of chlorine, fluorine, bromine, cyano and nitro.

5 Y¹ and the optional moiety Y² are independently selected from the group consisting of CH₂, O, NH, O(CO), NH(CO), O(CO)O, O(CS)O, NH(CO)O, NH(CS)O, NH(CO)NH, (CO)O and (CO)NH;

10 Z is an optional spacer unit selected from the group consisting of alkylene and oligoethylene glycolyl and combinations thereof, which may be unsubstituted or substituted; and

 W is a nucleosidic moiety containing a reporter group.

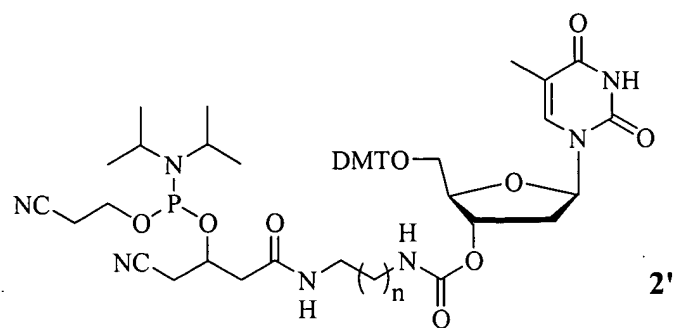
15 7. The phosphoramidite reagent of claim 6 wherein the reporter group is selected from the group of consisting of trityl, monomethoxy trityl and dimethoxy trityl (DMT).

 8. The phosphoramidite reagent of claim 6 wherein W is a nucleosidic moiety which comprises a 5'-DMT group as reporter group.

20 9. The phosphoramidite reagent of claim 6 wherein R is diisopropylamino.

25 10. The phosphoramidite reagent of claim 6 wherein X¹ and X² are independently selected from the group consisting of cyano, 4-nitrophenyl and phenylsulfonyl.

12. A phosphoramidite reagent having the formula (2'):



wherein n is an integer selected from 0 to 20.